

Project No. _____

Book No. _____

TITLE pmc9 / new primers.

106 11/22/94

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* AAT II * 1 : G C C A C C T G A C G T C T A A G A A A C C A
non du 31 mer TAT TAT e

* AAT II * 2 : G T T T C T T A G A C G T C A G G T G G C A C T T T T
non du 29 mer

* AAT II * 1 : G C C A C C U G A C G A C U A A G A A A C C A U T A T
du 31 mer

* AAT II * 2 : G T T T C T U A G A C G U C A G G U G G C A C U
du 29 mer TTT

Purpose To try to amplify pmc9 with these new pri

increased the annealing to 58° from 56° (previous exp.)

enzyme : Tag alone (10) primers : 1 non du } n
Tag + Deepvent (1 + 10) 2 du }
D.V. (0.5 + 1) 3 du - all

done in duplicate.

4 1 du + 2 no du

12 Rx / enzyme = Gen. the mix made

5. 2 du + 1 no du

Deepvent buffer was used this'ent.

Tag: buffer 60
dNTP 12
Temp. 2.4
enzyme 2.4
H₂O 403.2
480

Tube # (1 - 11)

200 µl dNTP
200 µl Temp
1 µl primers
2 µl Mg
Genes w. by

40 µl / Rx - added def. primer in 10 µl -

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Witnessed & Understood by me,

Date 11/24/94

Invented by

Date

Recorded by

11/28/94

St. Antanas

Page N _____

primers old } = 20x : ~~2.5~~ 10 + 10 ml of each primer at (100 µl)
 du }
 180 ml H₂O

primers du } = } equivalent amount of each
 new }
 other 2 different combos } 50 + 50 ml (10 µl each)
 1 du + other new du }

DV: buffer 60
 dNTP 12
 Temp 2.4
 enzyme 12.0 (1.0 µl/µl)
 H₂O 398.6
 480.0

20/µl

DV alone 0.5 µl 1.0
 buffer 60 60
 dNTP 12 12
 Temp 2.4 2.4
 enzyme 2.0 6.0
 H₂O 402.6 399.6

tube # 12 - 22

23 - 33

33 - 44

11, 22, 33 & 44 w/o any primers.

cycling: 94°, 3'

30 (94°, 30", 50°, 30", 72°, 2') → 4° soak

samples thrown out 12/19/94

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Date

11/23/94

Invented by

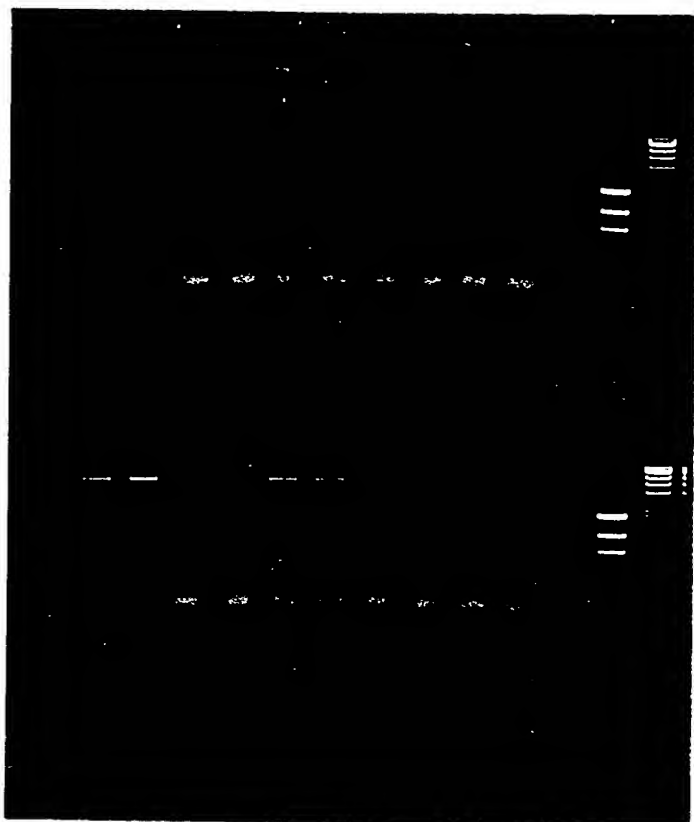
Recorded by

K. Sitarman

Date

11/23/94

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Tag:

4+ 4+ 0+ -/+ -/+ no primer

Tag + D.V. 1:0.01:

← Deep vent as usual
didn't reach →

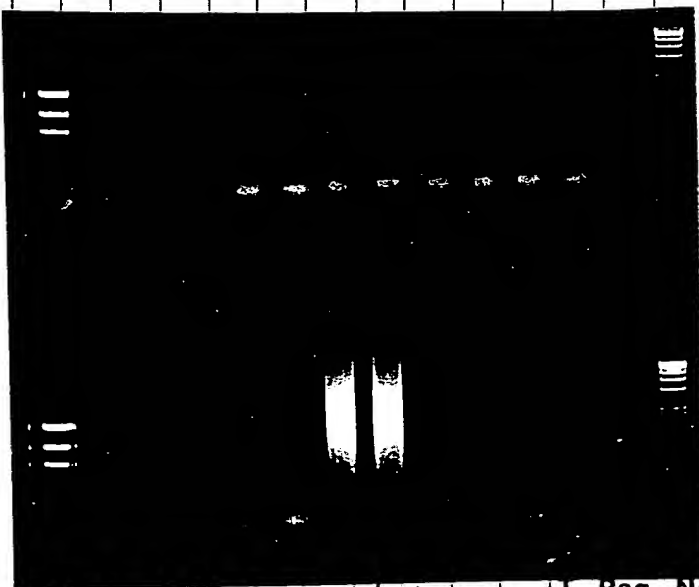
with old dv primer smears
for the first time.

Why more primer dimer
with dv than with
non dv?

Result:

- Even with Tag problem
with product at the
annealing temp.
- F/R - no dv product at
slightly with Tag, more
with T + D.V.
- old primer ^{also} at 50°, did
work with Tag.
- with Tag + D.V. primer
almost gone with old
primer new mispr
product with T + DV
- mismatch didn't work
at all.

D.V. 0.50



Witnessed & Understood by me,

Date

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